Comparative Studies of Transcytosis and Assembly of Secretory IgA in Madin-Darby Canine Kidney Cells Expressing Human Polymeric Ig Receptor[1]

Carol M. Tamer,* Michael E. Lamm,* Janet K. Robinson,* Janet F. Piskurich,[2]* and Charlotte S. Kaetzel[3]*†

*Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106; and †Departments of Pathology/Laboratory Medicine and Microbiology/Immunology, University of Kentucky, Lexington, KY 40536

Epithelial transport of polymeric IgA (pIgA) from its site of synthesis to the mucosal lumen is mediated by the polymeric Ig receptor (pIgR). During transcytosis, a disulfide bond forms between pIgR and pIgA, resulting in secretion of a covalently linked complex. To dissect further the intracellular processing and functions of pIgR, we have expressed the entire coding sequence of human pIgR cDNA in Madin-Darby canine kidney (MDCK) cells. Cloned transfected cells express human pIgR, as detected by immunofluorescence and by quantification of the cleaved extracellular domain of pIgR in culture supernatants. The function of transfected pIgR was confirmed by measuring vectorial transcytosis of ^{125}I-labeled pIgA and its disulfide bonding to pIgR. Species specificity of transcytosis was determined by comparing transport of human, rat, and mouse pIgA in MDCK cells expressing either human or rabbit pIgR. pIgA from all three species was transported by both human and rabbit pIgR, with rat pIgA being transported to the greatest extent in each case. However, disulfide bonding was observed only with human pIgR, and was found to occur mainly inside the cell. Our results suggest that conformational differences between human and rabbit pIgR may account for differences in disulfide bonding to pIgA, and show that efficient transcytosis of pIgA is correlated better with noncovalent than covalent binding to pIgR. *The Journal of Immunology,* 1995, 155: 707–714.

Secretory IgA, the main effector molecule of the mucosal immune system, protects the various epithelial surfaces of the body from invasion by inhaled and ingested pathogens including bacteria and viruses. Transport of polymeric Ig (pIg)[4] from its site of synthesis in the lamina propria to the mucosal lumen is mediated by the polymeric Ig receptor (pIgR).

The pIgR is synthesized as a transmembrane glycoprotein in the rough endoplasmic reticulum of mucosal epithelial cells (1–3). The receptor is further processed in the Golgi and sorted to the basolateral surface, one of the two plasma membrane domains of polarized epithelial cells (reviewed in Ref. 3). At the basolateral surface, the receptor noncovalently binds pIgA and is subsequently endocytosed and translocated to the apical surface of the cell. At the apical plasma membrane proteolytic cleavage occurs, and pIgA is released covalently coupled to the extracellular portion of pIgR as secretory IgA. The cleaved extracellular portion of pIgR, also known as secretory component (SC), has an approximate M_r of 80,000 and contains five Ig-like domains of 100 to 110 residues each (reviewed in Ref. 4). In the absence of IgA, pIgR is also cleaved at the apical plasma membrane and released as free SC. After the initial noncovalent interaction at the basolateral surface, most of the pIgA-pIgR complexes are stabilized through the formation of interchain disulfide bonds (5, 6). This disulfide bonding was shown to be a late event in hepatic transcytosis, presumably occurring at or near the bile canaliculus (7).

Received for publication February 9, 1995. Accepted for publication May 3, 1995.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grants CA-51998, AI-26449, and DK-43999. This work is part of a thesis submitted by C.M.T. in partial fulfillment for the degree of Master of Science, Case Western Reserve University.

[2] Current address: Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7295.

[3] Address correspondence and reprint requests to Dr. Charlotte S. Kaetzel, *Department of Pathology and Laboratory Medicine*, Markey Cancer Center, University of Kentucky, 800 Rose Street, Lexington, KY 40536-0093.

[4] Abbreviations used in this paper: pIg, polymeric Ig; SC, secretory component; MDCK, Madin-Darby canine kidney; dIgA, dimeric IgA.

0022-1767/95/$02.00

To understand better the kinetics and intracellular location and role of disulfide bond formation between pIgA and its receptor during transcytosis, we have expressed human pIgR cDNA in Madin-Darby canine kidney (MDCK) cells, a well-characterized, polarized epithelial cell line. MDCK cells expressing human pIgR provide a valuable model for studying epithelial transcytosis of pIgA, including disulfide bonding between receptor and ligand. Our results support the hypothesis that the physiologic role of disulfide bonding is to stabilize secreted pIgA-SC complexes rather than to facilitate transcytosis.

Materials and Methods

Cell culture

Wild-type MDCK cells (Type II) were obtained from the American Type Culture Collection (ATCC; Rockville, MD), and Type II MDCK cells stably transfected with cDNA for rabbit pIgR were provided by K. E. Mostov, University of California, San Francisco, (8). Cells were cultured in minimal essential medium (MEM) with nonessential amino acids, Earle's salts, 20 mM HEPES, pH 7.2, and 1 mM L-glutamine (Life Technologies, Gaithersburg, MD), 10% (v/v) fetal bovine serum (HyClone Laboratories, Inc., Logan, UT), and 100 U/ml penicillin, 100 μg/ml streptomycin, and 25 μg/ml amphotericin B (JRH Biosciences, Lenexa, KS).

Transfection of human pIgR cDNA in MDCK cells

We previously isolated from a human mammary gland lambda gt11 cDNA library two overlapping pIgR cDNA clones (hpIgR-1 and hpIgR-2) that together span the entire coding sequence of the human pIgR gene (9). Both cDNA clones were excised from the lambda gt11 vector with *Eco*RI and inserted into the *Eco*RI site of pBluescript II (Stratagene, La Jolla, CA). All restriction endonucleases used for DNA cloning were from Life Technologies. From the hpIgR-1 and hpIgR-2 templates, a cDNA subclone was constructed containing the entire pIgR coding sequence, 8 bp of 5′-untranslated sequence and 447 bp of 3′-untranslated sequence, as follows. To remove possible regulatory motifs in the 5′-untranslated region, a fragment spanning nucleotides (nt) −8 to +1054 (relative to the start site of translation) was prepared by PCR (Perkin-Elmer Corporation, Norwalk, CT), using hpIgR-1 cDNA as template. The oligodeoxynucleotide primers used in the PCR corresponded to nt −8 to +17 and the reverse complement of nt +1030 to +1054 of human pIgR cDNA. To facilitate subcloning with *Eco*RI, the sequence ACGTACGAATTC was included at the 5′ end of the upper primer. The DNA fragment was then digested with *Eco*RI, which cleaves just before the sequence AATTC of the upper primer, and *Bcl*I, which cleaves at position +932 of the pIgR coding sequence. A second DNA fragment was prepared from hpIgR-2 cDNA. Because *Bcl*I does not cleave methylated DNA from plasmids grown in conventional *Escherichia coli* strains, the hpIgR-2 cDNA/pBluescript II plasmid was transformed into the *dam*⁻ *E. coli* strain DM1 (Life Technologies) before further subcloning steps. Purified hpIgR-2 plasmid DNA was digested with *Bcl*I, which cleaves at position +932 of the pIgR coding sequence, and *Eco*RI, which cleaves at the 3′ end of the hpIgR-2 insert, containing 447 bp of 3′-untranslated sequence beyond the coding sequence. The two DNA fragments were ligated at their *Bcl*I sites and inserted into the *Eco*RI site of pBluescript II. To determine that no mutations were introduced into the DNA during PCR and subcloning procedures, the entire insert was sequenced by the dideoxy chain-termination method (10), and its sequence compared with the original hpIgR-1 and hpIgR-2 cDNA clones (9). The entire cDNA fragment was then excised from pBluescript II with *Eco*RI, and subcloned using *Eco*RI–*Hin*dIII linkers into the *Hin*dIII site of pCB6 (a gift from K. E. Mostov), an expression vector that contains the sodium butyrate-inducible cytomegalovirus early promoter (11), the human growth hormone polyadenylation site, and the *neo* resistance gene.

MDCK cells were transfected with pCB6 containing the human pIgR cDNA via lipofectin (Life Technologies). Cells were grown to ~50% confluence in 100-mm tissue culture plates. For each plate, 40 μl (40 μg) of lipofectin and 20 μg of DNA were mixed with 1 ml serum-free medium and kept at room temperature for 10 to 15 min. Before transfection, cells were washed twice with Dulbecco's PBS (10 mM Na_2HPO_4, pH 7.4, 150 mM NaCl, 4.1 mM KCl, 0.5 mM $CaCl_2$, 0.9 mM $MgCl_2$) and once with serum-free medium. The lipofectin-DNA mixture was then gently added and incubated for 5 h at 37°C, after which the medium was removed and replaced with serum-containing medium. The following day all media were replenished. Two days later, the neomycin analogue Geneticin (G418 sulfate; Life Technologies) was added at 0.9 mg/ml, a concentration that had been determined in preliminary experiments to kill > 99% of untransfected cells. Cells were treated with Geneticin for 10 days and cloned by limiting dilution.

Comparisons between the sequences of human (9, 12, 13) and rabbit (14) pIgR were made with the MegAlign program (DNASTAR Inc., Madison, WI).

Expression of human pIgR in transfected MDCK cells

Geneticin-resistant clones were tested for expression of human pIgR by measurement of SC in culture supernatants. SC was quantified in culture supernatants by ELISA as previously described (15). Briefly, microtiter plates were coated with guinea pig antiserum to human SC, and incubated either with human SC diluted in culture medium, as standards, or with experimental samples. Bound SC was detected by incubating with rabbit antiserum to human SC, followed by alkaline phosphatase-conjugated goat Abs to rabbit IgG (Boehringer Mannheim, Indianapolis, IN) and Sigma 104 phosphatase substrate (Sigma Chemical Co., St. Louis, MO). Cells were trypsinized and counted with a hemacytometer. Data were calculated as nanogram of SC/10^5 cells/24 h. To test for inducibility of the CMV promoter driving pIgR expression, cells were treated with 1 mM sodium butyrate for 24 h before collection of culture supernatants and measurement of SC.

Expression of cell-associated pIgR was determined by immunofluorescence with the rabbit antiserum to human SC described above for ELISA. Cloned MDCK cells were plated in 8-well chamber slides (Nunc, Inc., Naperville, IL) and treated for 24 h with 1 mM sodium butyrate. Cells were rinsed with PBS and fixed in acetone for 1 min at room temperature. Cells were then rehydrated in PBS, washed three times for 5 min with PBS, and incubated for 40 min with rabbit antiserum to human SC, diluted 1:100 in PBS containing 5% fetal bovine serum. Normal rabbit serum was added to the negative controls. Cells were washed as before and incubated for 30 min with FITC-conjugated goat Abs to rabbit IgG (Southern Biotechnology Associates, Inc., Birmingham, AL), diluted 1:50 in PBS/5% fetal bovine serum. After the cells were washed with PBS, the chambers were removed and the slides were mounted with Fluoromount-G (Southern Biotechnology Associates, Inc.) and viewed with a Leitz Orthoplan microscope with epifluorescence.

Isolation of human, rat, and mouse pIgA

To compare the species specificity of pIgA transcytosis by MDCK cells expressing human or rabbit pIgR, monoclonal human, rat, and mouse IgAs were isolated. The human IgAs were purified from plasma of three patients (designated A, B, and C) with IgA multiple myeloma. Sera were obtained by clotting the plasma and then precipitated with ammonium sulfate at 50% saturation to isolate the Ig fraction. Rat IgA from ascites fluid induced by LO-DNP-64 hybridoma cells was provided by J.-P. Vaerman, Catholic University of Louvain, Brussels, Belgium (16). Rat IR22 myeloma IgA from ascites (Zymed Laboratories, Inc., South San Francisco, CA) and R3–30 hybridoma IgA from tissue culture supernatants (PharMingen, San Diego, CA) were purified as described below. Mouse IgA was isolated from ascites fluid of mice carrying MOPC-315 myeloma cells (17) (ATCC) or TEPC-15 myeloma cells (17) (provided by S. Emancipator, Case Western Reserve University, Cleveland, OH). Dimers and higher polymers of IgA were separated from monomeric IgA by gel filtration (18) and purity was confirmed by SDS-PAGE under nonreducing conditions. Before gel filtration, ascites fluid was delipidated with Seroclear reagent (Calbiochem, La Jolla, CA) and filtered. Purified dimeric IgA (dIgA) and pIgA were labeled with ^{125}I (Amersham Corp., Arlington Heights, IL) via lactoperoxidase (19) (Calbiochem) to a specific activity of ~5×10^7 cpm/μg.

Transcytosis of pIgA

MDCK cells expressing human pIgR or rabbit pIgR were grown to confluence on 30-mm nitrocellulose filters (Millicell-HA; Millipore Corp., Bedford, MA), inserted in 35-mm tissue culture wells. Tightness of

monolayers was tested by measuring transmembrane electrical resistance with a Millicell-ERS meter (Millipore). The filters were rinsed with cold Dulbecco's PBS without BSA, and incubated with HEPES buffer (50 mM HEPES, pH 7.4, 118 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 10 mM $CaCl_2$, 8.8 mM dextrose) containing 1% (w/v) BSA, which had been treated at 56°C for 30 min to inactivate endogenous proteases. One milliliter of cold HEPES/BSA buffer was added to the apical chamber, and 1.5 ml of cold HEPES/BSA buffer containing 1.5 μg of rat, mouse, or human ^{125}I-labeled pIgA was added to the basolateral chamber. To allow binding of ^{125}I-labeled pIgA, filters were incubated on ice for 3 h. The filters were washed six times with cold Dulbecco's PBS containing 0.1% BSA, then warmed by adding 37°C HEPES buffer without BSA to the apical and basolateral chambers. The filters were placed in a 37°C incubator and samples of apical culture supernatants were collected at varying time points. The amount of ^{125}I-labeled pIgA transcytosed was determined by precipitating an aliquot of apical supernatant with cold 10% TCA. The remainder of the apical supernatant was used, where designated, for analysis of disulfide bonding between dIgA and SC.

Analysis of disulfide bonding between SC and dIgA

Transcytosis of human, rat, and mouse ^{125}I-labeled dIgA across monolayers of MDCK cells expressing human or rabbit pIgR was conducted as described above, and apical supernatants were collected after 2 h. To block subsequent disulfide bonding, iodoacetamide (Sigma Chemical Co.), in 1 M Tris, pH 8.0, was added to a final concentration of 25 mM as previously described (7). To determine the extent of spontaneous disulfide bond formation between SC and dIgA that might occur in the apical medium after transcytosis, we collected apical supernatants from control cultures that were incubated in the absence of dIgA for 2 h at 37°C. Since pIgR is transcytosed, cleaved, and SC released in the absence as well as in the presence of pIgA (20), these control supernatants should contain approximately the same concentration of SC as supernatants from pIgA-treated cultures. The control supernatants were incubated in vitro with ^{125}I-labeled dIgA. The amounts of dIgA added to the control supernatants were calculated to approximate the amounts of dIgA typically transcytosed in a 2-h period by each respective receptor: 594 pg of human dIgA, 985 pg of rat dIgA, or 343 pg of mouse dIgA were added to 1 ml of apical supernatant containing human SC; 1484 pg of human dIgA, 2956 pg of rat dIgA, or 800 pg of mouse dIgA were added to 1 ml of apical supernatant containing rabbit SC. Control and experimental apical supernatants were then immunoprecipitated with anti-SC as previously described (7). Briefly, rabbit Abs to human SC (which cross-react with rabbit SC; data not shown) were bound to Protein G-Sepharose beads (Pharmacia Biotechnology, Piscataway, NJ) at a ratio of 40 μl antiserum/100 μl beads. Fifty microliters of the coated beads were then added to 800 μl of the apical supernatants and rocked overnight at 4°C. The beads were recovered by centrifugation, boiled with SDS-PAGE sample buffer, and analyzed by nonreducing SDS-PAGE with 3% stacking and 5% separating gels (21). The relative migration of ^{125}I-labeled dIgA was determined by autoradiography. The extent of disulfide bonding between SC and dIgA was determined by densitometry of the autoradiograms as previously described (7), and observing a difference in M_r of 80,000 between dIgA covalently bound to SC and free dIgA (or dIgA that had been noncovalently bound to SC, which dissociates during SDS-PAGE).

Results

Characterization of clones stably transfected with human pIgR cDNA

To develop a system for studying transcytosis of pIgA by human pIgR, MDCK cells were stably transfected with the pCB6 plasmid vector, in which expression of human pIgR cDNA is driven by the CMV early promoter. Two criteria were used to assess pIgR expression in stable transfectants. We previously demonstrated that release of SC (the cleaved extracellular domain of pIgR) into culture supernatants is proportional to total cellular production of pIgR (9, 15). Transfectants were first screened for pIgR expression by measuring SC levels in culture supernatants. Clone 4 and clone 17 were shown to release 5 to 10 ng of SC/10^5 cells/24 h (Fig. 1), while no SC was detected in culture supernatants from untransfected MDCK cells (data not shown). Treatment of cells with 1 mM sodium butyrate increased the release of SC up to 10-fold (Fig. 1), presumably due to up-regulation of the CMV promoter (11). Second, the expression of cell-associated pIgR by transfected cells treated with sodium butyrate was determined by immunofluorescence. Both clone 4 and clone 17 cells were shown to uniformly express human SC (Fig. 2). The greater intensity of immunofluorescence in clone 4 cells correlates with the increased release of SC (compare Figs. 1 and 2).

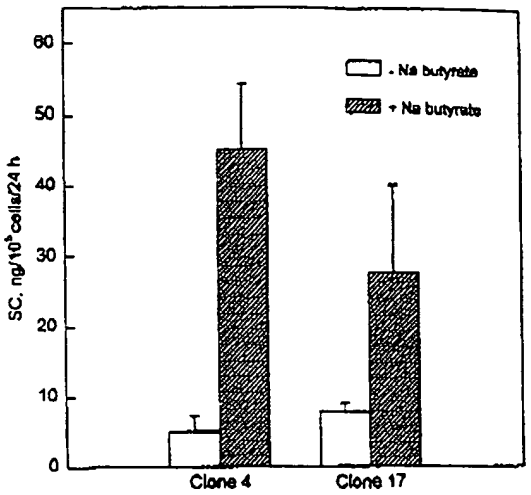

FIGURE 1. Expression of human pIgR in MDCK cells stably transfected with the pCB6 expression vector containing human pIgR cDNA. Two independent clones (clone 4 and clone 17) were isolated by limiting dilution, grown to confluence, and cultured for 24 h in the presence and absence of 1 mM sodium butyrate, which up-regulates the CMV promoter. Supernatants were collected for quantification of SC (the cleaved extracellular domain of pIgR) by ELISA. Data are expressed as mean ± SD ng SC/10^5 cells/24 h (n = 4).

Human pIgR expressed in MDCK cells transports human, rat, and mouse pIgA

The function of human pIgR in transfected MDCK cells was tested by monitoring transcytosis of dIgA. Clone 4 and clone 17 cells were grown to confluence on nitrocellulose filters and transcytosis of radiolabeled human (patient A), rat (LO-DNP-64), and mouse (MOPC-315) dIgA was measured. Both clone 4 and clone 17 cells transported ^{125}I-labeled human, rat, and mouse dIgA from the basolateral surface of the cells into the apical medium (Fig. 3). In four replicate experiments the rat dIgA was always the most efficiently transcytosed. Clone 4 cells transcytosed more dIgA of each species than did clone 17 cells, consistent with their higher expression of pIgR. To determine

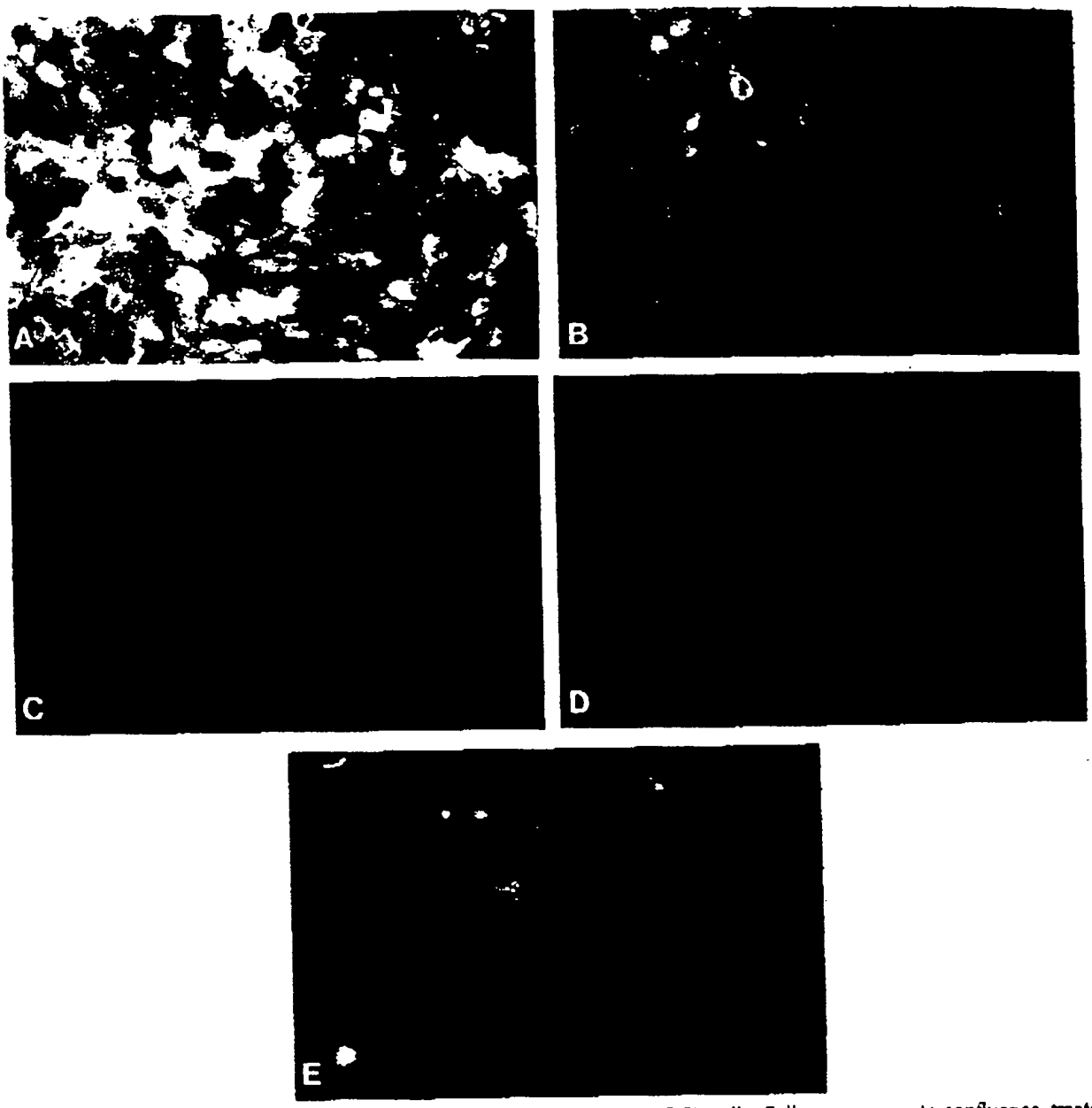

FIGURE 2. Uniform expression of human pIgR by cloned, transfected MDCK cells. Cells were grown to confluence, treated for 24 h with sodium butyrate, and assayed for cell-associated pIgR expression by immunofluorescence. Clone 4 cells are shown in *A*, *C*, and *E*, and clone 17 cells are shown in *B* and *D*. Cells were fixed with acetone and incubated with rabbit antiserum to human SC (*A*, *B*, and *E*) or normal rabbit serum (*C* and *D*), followed by FITC-conjugated goat Abs to rabbit IgG. Clone 4 (*A*) and clone 17 (*B*) cells uniformly expressed pIgR, although the intensity of staining was greater for clone 4 cells. At higher magnification of clone 4 cells (*E*), one can better appreciate the subcellular localization of pIgR. Greatest staining is observed in the perinuclear region, presumably reflecting newly synthesized pIgR in the secretory pathway. Magnification, ×144 (*A–D*) and ×289 (*E*).

directly whether there is a correlation between the expression of pIgR and the amount of dIgA transcytosed, clone 4 cells were treated as described above in the presence and absence of sodium butyrate for 24 h. Cells treated with sodium butyrate transcytosed twice as much rat dIgA to the apical surface as did untreated cells (data not shown). Clone 4 cells treated with sodium butyrate were therefore used for all subsequent experiments.

To test for species specificity of transcytosis, the extent of transport of human, rat, and mouse pIgA by MDCK cells expressing either human or rabbit pIgR was determined (Table I). All IgAs were transported by both human

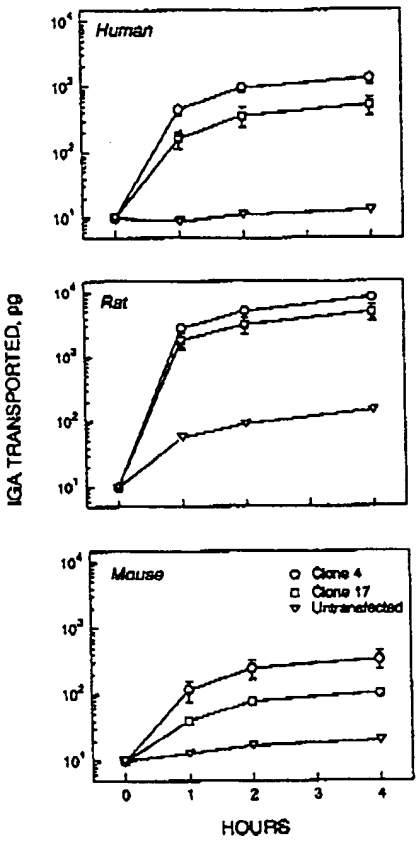

FIGURE 3. Human pIgR mediates transcytosis of human, rat, and mouse dIgA in transfected MDCK cells. Clone 4, clone 17, and untransfected MDCK cells were grown to confluence on nitrocellulose filters and treated for 24 h with sodium butyrate to up-regulate pIgR expression. ^{125}I-labeled human, rat, or mouse dIgA was allowed to bind to the basolateral surface of the cells for 3 h at 0°C. Cultures were then incubated at 37°C to allow transport of dIgA, and apical culture supernatants were collected at varying time points. Transcytosed ^{125}I-labeled dIgA was detected by TCA-precipitable ^{125}I. Data are expressed as mean ± SD pg dIgA transported (n = 4). (○) Clone 4 cells; (□) clone 17 cells; (▽) untransfected MDCK cells.

and rabbit pIgR, and as previously observed, the rat IgAs were the most efficiently transcytosed by both human and rabbit pIgR (compare Fig. 3 and Table I). In addition to interspecies variability, IgAs from within the same species varied in their efficiency of transcytosis. Some of this variability in transcytosis may be attributed to the relative amounts of dimers vs higher polymers of IgA, a characteristic that varied considerably among sources of IgA within each species (Table I). Despite this variability in extent of transcytosis, clear differences were observed between human and rabbit pIgR with regard to disulfide bonding to pIgA (see below).

Table I. *Transcytosis of human, rat, and mouse pIgA by human and rabbit pIgR*

Source of IgA[b]	Transcytosis (pg/4 h)[a] Source of pIgR: Human	Rabbit	None[c]
Human[d]			
A (dimers)	207	1916	14
B (dimers)	149	1607	16
C (polymers)	2739	745	127
Rat			
IR22 (polymers)	5497	3168	106
LO-DNP-64 (dimers)	2508	5335	138
R3-30 (polymers)	5214	2638	530
Mouse			
TEPC-15 (dimers)	1088	187	13
MOPC-315 (dimers)	80	276	16

[a] Transcytosis of ^{125}I-labeled pIgA by filter-grown MDCK cells was determined as described in *Materials and Methods*. Values represent the average of duplicate filters. These data are representative of four transcytosis experiments.
[b] IgAs from three species were purified, and pIgA was separated from monomeric IgA by gel filtration. Depending on the composition of each individual IgA, the purified fraction was composed mainly of IgA dimers or a mixture of dimers and higher polymers, as indicated. Purified IgA was labeled with ^{125}I to a specific activity of 5.2 ± 1.6 × 10^7 cpm/μg (mean ± SD for all eight purified IgA preparations).
[c] Untransfected MDCK cells.
[d] Human pIgA was isolated from the sera of three patients (designated A, B, and C) with IgA multiple myeloma.

Human, but not rabbit, pIgR forms disulfide bonds with dIgA from three species

To examine whether disulfide bond formation occurs between pIgR and dIgA during transport, apical supernatants were collected from MDCK cells expressing human or rabbit pIgR following transport of ^{125}I-labeled human, rat, and mouse dIgA. The extent of disulfide bonding between dIgA and SC was determined by observing a m.w. shift in transported ^{125}I-labeled dIgA (Fig. 4). Human dIgA has an apparent M_r of 340,000 on nonreducing SDS-PAGE; when SC (M_r 80,000) is covalently bonded to human dIgA, there is a shift in its apparent M_r to 420,000. In contrast, rat and mouse dIgA have an apparent M_r of 240,000 on nonreducing SDS-PAGE, because the Ig light chains are not covalently bonded to the heavy chains and thus dissociate in the presence of SDS (22). When rat and mouse dIgA are disulfide bonded to SC, their apparent M_r shifts to 320,000 (7).

Although all three species of dIgA were transcytosed by both human and rabbit pIgR, only human pIgR became disulfide bonded to dIgA (Fig. 4). Disulfide bonding was not 100% efficient, similar to observations with rat dIgA transcytosed by rat hepatocytes (7). The extent of disulfide bonding in vitro in the control samples (Fig. 4, human pIgR, *lane b*) was much lower than in cell-assembled secretory IgA (Fig. 4, human pIgR, *lane c*), regardless of the source of dIgA, indicating that the formation of disulfide bonds between dIgA and pIgR is primarily a cell-associated event. No correlation was seen between disulfide bond formation and the amount of dIgA transcytosed (compare Table I and Fig. 4), suggesting that disulfide bonding does not facilitate transcytosis.

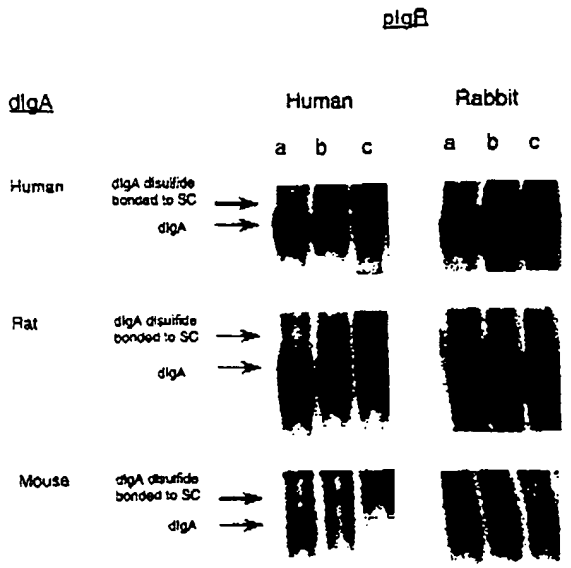

FIGURE 4. Human, but not rabbit, pIgR forms disulfide bonds with dIgA from three species. MDCK cells expressing human pIgR (clone 4) or rabbit pIgR were grown to confluence on nitrocellulose filters and treated for 24 h with sodium butyrate to up-regulate pIgR expression. Transcytosis of ^{125}I-labeled human (patient A), rat (LO-DNP-64), or mouse (MOPC-315) dIgA (purified dimers) was performed as described in the legend for Figure 3. After 2 h, apical culture supernatants were collected and incubated with 25 mM iodoacetamide to prevent any further disulfide bonding in vitro. After immunoprecipitation with Abs to SC, the samples were analyzed by nonreducing SDS-PAGE and autoradiography. (*lane a*) Starting material (^{125}I-labeled dIgA). (*lane b*) Control apical supernatants (containing human or rabbit SC) incubated in vitro with ^{125}I-labeled dIgA. (*lane c*) Apical supernatants containing transcytosed ^{125}I-labeled dIgA-SC complexes. Slower migrating bands, representing covalently bonded dIgA-SC complexes, are observed only in supernatants from cells expressing human pIgR.

Discussion

Secretory IgA has a crucial role in host defense at mucosal sites. Extensive studies have examined the transport of pIgA from its site of synthesis by plasma cells in the lamina propria across the epithelial lining into the mucosal secretions. After the initial noncovalent interaction of pIgA with pIgR at the basolateral surface of the epithelial cells, the receptor-ligand complex is endocytosed. During its passage to the apical surface and before release of secretory IgA into the external secretions, pIgA becomes covalently associated with pIgR. In rat liver, disulfide bond formation between dIgA and pIgR appears to occur late in the transcytotic pathway, and does not facilitate transcytosis (7).

Here we have developed a system, using MDCK cells transfected with human pIgR cDNA, to study covalent assembly and transcytosis of secretory IgA. We have shown that human pIgR, like rabbit pIgR, can transport human, rat, and mouse pIgAs. However, disulfide bond formation with these three kinds of pIgA was observed for human but not rabbit pIgR. Furthermore, the extent of disulfide bonding between human pIgR and rat dIgA was similar to the extent of disulfide bonding between rat pIgR and rat pIgA occurring in rat liver in vivo (7). Therefore, the transfected MDCK cells provide a good system to study disulfide bonding, and human pIgR appears to be comparable to rat pIgR with regard to its ability to form disulfide bonds to pIgA.

To determine whether there was a correlation between disulfide bonding to pIgR and transcytosis of IgA, we compared the extent of disulfide bonding of representative human (patient A), rat (LO-DNP-64), and mouse (MOPC-315) dIgAs (Fig. 4) with the extent of transcytosis of the same dIgAs (Table I). Although rabbit pIgR did not disulfide bond with any of these IgAs, the efficiency of transcytosis was as great or greater with rabbit pIgR than with human pIgR (Table I). These results are consistent with the hypothesis that disulfide bonding does not facilitate transcytosis, but may play an important physiologic role in stabilizing pIgA-SC complexes that have been secreted (1, 7, 23).

The first Ig-like domain of rabbit (24, 25) and human (26–28) pIgR mediates its initial noncovalent association with pIgA. A 23-amino acid sequence within domain 1, which is highly conserved across species, is necessary for pIgA binding (reviewed in Ref. 4). Disulfide bonding with pIgA, however, occurs at a highly conserved cysteine residue in domain 5 of pIgR (5, 6). To examine whether differences in the structure of pIgR may account for species differences in noncovalent vs covalent bonding to pIgA, we compared homologies in domains 1 and 5 of human and rabbit pIgR. The sequence similarity between human and rabbit pIgR within domain 1 is 59%, and increases to 83% within the 23-amino acid noncovalent "pIg-binding site." Noncovalent binding of human, rat, and mouse pIgA might therefore be expected to be similar for human and rabbit pIgR. In contrast, sequence similarity between human and rabbit pIgR is only 40% within domain 5, which mediates covalent binding to pIgA. Although the critical cysteine residue in domain 5 is conserved between human and rabbit pIgR, it is possible that differences in the overall structure of this domain may result in differences in alignment with human, rat, and mouse pIgA, thus permitting disulfide bonding with human pIgR while preventing disulfide bonding with rabbit pIgR.

While no disulfide bonding with rabbit pIgR was seen with human, rat, and mouse pIgA, MDCK cells expressing rabbit pIgR can form disulfide bonds with a chimeric pIgA molecule in which the constant regions of the α heavy chains are derived from rabbit IgA (29). Therefore, the absence of disulfide bonding between human, rat, and mouse pIgA and rabbit pIgR in our experiments most

likely results from a structural misalignment between domain 5 of rabbit pIgR and the α heavy chains of pIgA, rather than from a functional deficiency of the transfected MDCK cells or an inherent inability of rabbit pIgR to form disulfide bonds with pIgA. This hypothesis is consistent with the observation that the f subclass of rabbit IgA is covalently bound to SC in natural rabbit secretory IgA (30). Taken together, our results suggest that conservation of the noncovalent pIg-binding site may account for similarities in transcytosis mediated by human and rabbit pIgR, and show that efficient transcytosis of pIgA is correlated better with noncovalent than covalent binding to pIgR.

Several pIgAs from three species were used to determine specificity of transcytosis by human and rabbit pIgR. We showed that even though pIgAs from all three species were transported by both human and rabbit pIgR, rat pIgA was most efficiently transcytosed. In addition, significant variation in transcytosis efficiency was observed between pIgAs from the same species (Table I). Depending on the choice of pIgA within the same species, one would expect some structural differences due to variations in post-translational modifications (e.g. glycosylation, ratio of dimers to higher polymers) of each pIgA (31). Comparative studies of the transport of pIgA from blood to bile have shown that the mechanisms of secretory immunity can vary among different mammals. For example, while rats and rabbits transported human and rat pIgA from blood to bile with similar kinetics, guinea pigs and sheep could not transport any class of human or rat Ig but could transport their own pIgA (32). These results along with our data emphasize that generalizations from one species to another should not be made without supporting evidence.

In conclusion, the system we have established should be valuable for dissecting the intracellular processing and functions of human pIgR in transcytosis of pIgA. Moreover, understanding the noncovalent as well as the covalent interactions of pIgA and pIgR could be important for optimally engineering Ag-specific secretory IgAs capable of being used therapeutically to combat infection. Recent experiments have shown that administration of IgA mAbs that lacked secretory component provided protection of only short duration, probably due to degradation of IgA by mucosal proteases (33). It may therefore be important for passive immunotherapy with IgA to provide covalently linked pIgA-SC complexes.

References

1. Brandtzaeg, P. 1985. Role of J chain and secretory component in receptor-mediated glandular and hepatic transport of immunoglobulins in man. *Scand. J. Immunol. 22:111.*
2. Brandtzaeg, P., P. Krajci, M. E. Lamm, and C. S. Kaetzel. 1994. Epithelial and hepatobiliary transport of polymeric immunoglobulins. In *Handbook of Mucosal Immunology.* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. R. McGhee, and J. Bienenstock, eds. Academic Press, San Diego, CA, p. 113.
3. Mostov, K. E. 1994. Transepithelial transport of immunoglobulins. *Annu. Rev. Immunol. 12:63.*
4. Piskurich, J. F., M. H. Blanchard, K. R. Youngman, J. A. France, and C. S. Kaetzel. 1995. Molecular cloning of the mouse polymeric Ig receptor: functional regions of the molecule are conserved among five mammalian species. *J. Immunol 154:1735.*
5. Cunningham-Rundles, C., and M. E. Lamm. 1975. Reactive half-cystine peptides of the secretory component of human exocrine immunoglobulin A. *J. Biol. Chem. 250:1987.*
6. Fallgreen-Gebauer, E., W. Gebauer, A. Bastian, H. D. Kratzin, H. Eiffert, B. Zimmermann, M. Karas, and N. Hilschmann. 1993. The covalent linkage of secretory component to IgA: structure of sIgA. *Biol. Chem. Hoppe-Seyler 374:1023.*
7. Chintalacharuvu, K. R., A. S. Tavill, L. N. Louis, J.-P. Vaerman, M. E. Lamm, and C. S. Kaetzel. 1994. Disulfide bond formation between dimeric immunoglobulin A and the polymeric immunoglobulin receptor during hepatic transcytosis. *Hepatology 19:162.*
8. Mostov, K. E., and D. L. Deitcher. 1986. Polymeric immunoglobulin receptor expressed in MDCK cells transcytoses IgA. *Cell 46:613.*
9. Piskurich, J. F., J. A. France, C. M. Tamer, C. A. Willmer, C. S. Kaetzel, and D. M. Kaetzel. 1993. Interferon-γ induces polymeric immunoglobulin receptor mRNA in human intestinal epithelial cells by a protein synthesis-dependent mechanism. *Mol. Immunol. 30:413.*
10. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA 74: 5463.*
11. Chang, C.-P., C. L. Malone, and M. F. Stinski. 1989. A human cytomegalovirus early gene has three inducible promoters that are regulated differentially at various times after infection. *J. Virol. 63:281.*
12. Eiffert, H., E. Quentin, J. Decker, R. N. Eisenman, M. Hufschmidt, D. Klingmuller, M. H. Weber, and N. Hilschmann. 1984. The primary structure of the human free secretory component and the arrangement of the disulfide bonds. *Hoppe-Seyler's Z. Physiol. Chem. 365:1489.*
13. Krajci, P., K. H. Grzeschik, A. H. M. Geurts van Kessel, B. Olaisen, and P. Brandtzaeg. 1991. The human transmembrane secretory component (poly-Ig receptor): molecular cloning, restriction fragment length polymorphism and chromosomal sublocalization. *Hum. Genet. 87:642.*
14. Mostov, K. E., M. Friedlander, and G. Blobel. 1984. The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. *Nature 308:37.*
15. Chintalacharuvu, K. R., J. F. Piskurich, M. E. Lamm, and C. S. Kaetzel. 1991. Cell polarity regulates the release of secretory component, the epithelial receptor for polymeric immunoglobulins, from the surface of HT-29 colon carcinoma cells. *J. Cell. Physiol. 148:35.*
16. Rits, M., F. Cormont, H. Bazin, R. Meykens, and J.-P. Vaerman. 1986. Rat monoclonal antibodies. VI. Production of IgA-secreting hybridomas with specificity for the 2,4-dinitrophenyl (DNP) hapten. *J. Immunol. Methods 89:81.*
17. Potter, M. 1972. Immunoglobulin-producing tumors and myeloma proteins of mice. *Physiol. Rev. 52:631.*
18. Kaetzel, C. S., J. K. Robinson, K. R. Chintalacharuvu, J.-P. Vaerman, and M. E. Lamm. 1991. The polymeric immunoglobulin receptor (secretory component) mediates transport of immune complexes across epithelial cells: a local defense function for IgA. *Proc. Natl. Acad. Sci. USA 88:8796.*
19. Thorell, J. I., and B. G. Johansson. 1971. Enzymatic iodination of polypeptides with ^{125}I to high specific activity. *Biochim. Biophys. Acta 251:363.*
20. Kuhn, L. C., and J. Krachenbuhl. 1982. The sacrificial receptor: translocation of polymeric IgA across epithelia. *Trends. Biochem. Sci. 7:299.*
21. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227:680.*
22. Chintalacharuvu, K. R., M. E. Lamm, and C. S. Kaetzel. 1993. Unstable inter-H chain disulfide bonding and noncovalently associated J chain in rat dimeric IgA. *Mol. Immunol. 30:19.*
23. Brown, W. R., R. W. Newcomb, and K. Ishizaka. 1970. Proteolytic degradation of exocrine and serum immunoglobulins. *J. Clin. Invest. 49:1374.*
24. Frutiger, S., G. J. Hughes, W. C. Hanly, M. Kingzette, and J. Jaton. 1986. The amino-terminal domain of rabbit secretory component is

responsible for noncovalent binding to immunoglobulin A dimers. *J. Biol. Chem. 261:16673.*

25. Coyne, R. S., M. Siebrecht, M. C. Peitsch, and J. E. Casanova. 1994. Mutational analysis of polymeric immunoglobulin receptor/ligand interactions: evidence for the involvement of multiple complementarity-determining region (CDR)-like loops in receptor domain I. *J. Biol. Chem. 269:31620.*
26. Bakos, M.-A., A. Kurosky, and R. M. Goldblum. 1991. Characterization of a critical binding site for human polymeric Ig on secretory component. *J. Immunol. 147:3419.*
27. Bakos, M.-A., A. Kurosky, E. W. Czerwinski, and R. M. Goldblum. 1993. A conserved binding site on the receptor for polymeric Ig is homologous to CDR1 of Ig V_K domains. *J. Immunol. 151:1346.*
28. Bakos, M.-A., S. G. Widen, and R. M. Goldblum. 1994. Expression and purification of biologically active domain I of the human polymeric immunoglobulin receptor. *Mol. Immunol. 31:165.*
29. Schoeiderman, R. D., W. C. Hanly, and K. L. Knight. 1989. Expression of 12 rabbit IgA c-alpha genes as chimeric rabbit-mouse IgA antibodies. *Immunology 86:7561.*
30. Knight, K. L., M. L. Vetter, and T. R. Malek. 1975. Distribution of covalently bound and non-covalently bound secretory component on subclasses of rabbit secretory IgA. *J. Immunol. 115:595.*
31. Hickman, S., and J. L. Theodorkis. 1985. Characterization of MOPC-315 IgA oligosaccharide processing intermediates. *Biochem. Biophys. Res. Commun. 128:586.*
32. Hall, J. G., L. A. Gyure, and A. W. R. Payne. 1980. Comparative aspects of the transport of immunoglobulin A from blood to bile. *Immunology 41:899.*
33. Kraehenbuhl, J.-P., and M. R. Neutra. 1992. Molecular and cellular basis of immune protection of mucosal surfaces. *Physiol. Rev. 72:853.*